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Retroviral gene transfer into human hematopoietic cells: an in vitro kinetic study.

Briones J, Puig T, Limon A, Petriz J, Garcia J, Barquinero J

Department of Cryobiology and Cell Therapy, Institut de Recerca Oncologica, L'Hospitalet, Barcelona, Spain.

BACKGROUND AND OBJECTIVE: Successful gene therapy applications require optimized strategies to increase gene transfer efficiency into hematopoietic progenitor cells (HPCs) with long-term repopulating ability. One of the issues that needs to be clarified is how hematopoietic cells proliferate, differentiate and express the transgene after each cycle of transduction. We investigated the kinetics of cell expansion, CD34 antigen expression and transduction efficiency of human hematopoietic cells in culture conditions commonly used in retroviral gene transfer protocols. **DESIGN AND METHODS:** Purified CD34+ cells from cord blood (n=5) or leukapheresis products (n=9) and a retroviral vector encoding an enhanced version of the green fluorescent protein (EGFP) were used. Target cells were exposed daily to vector-containing supernatants and a combination of interleukin 3 (IL-3), interleukin 6 (IL-6), stem cell factor (SCF) and Flt3-ligand (FL). Cell samples were harvested from the cultures and analyzed at 24 hour intervals for seven consecutive days. **RESULTS:** We found that CD34+ cells proliferated and differentiated under our culture conditions. The number of genetically modified cells increased after each cycle of transduction. Median numbers of cells positive for both CD34 and EGFP increased steadily over the culture period, but after day four most of the EGFP+ cells had a low CD34 expression. **INTERPRETATION AND CONCLUSIONS:** Culturing and transducing CD34+ cells for longer periods of time under these conditions might be detrimental for ex vivo gene transfer applications since the transduced cells are likely to have a decreased potential for long-term engraftment and repopulation in vivo.

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